

Appl. No. 09/743,684
Amdt. Dated January 30, 2006
Reply to Office Action of July 28, 2005

Attorney Docket No. 89188.0130
Customer No.: 26021

Amendments to the Specification:

Please replace the three paragraphs beginning at page 3, line 9 with the following rewritten paragraphs:

In yet another embodiment, the isolated polypeptide specifically binds to an antibody raised against Saposin B. In a preferred embodiment, the polypeptide comprises an amino acid sequence substantially identical to that shown in SEQ ID NO:12 beginning at position 72. In a most preferred embodiment, the polypeptide comprises at least 5 contiguous amino acids, or conservatively modified variants thereof, said contiguous amino acids having an amino acid sequence as shown in SEQ ID NO:12, beginning at position 72.

In still another embodiment, the isolated polypeptide comprises R-DVCQD-R' (SEQ ID NO:44); wherein R is from 0 to about 6 contiguous amino acids; and wherein R' is from 0 to about 59 contiguous amino acids. In a preferred embodiment, the polypeptide comprises R-XDVCQD-R' (SEQ ID NO:45); wherein R is selected from the group consisting of Aa₁-Aa₂-Aa₃-Aa₄-Aa₅, Aa₂-Aa₃-Aa₄-Aa₅, Aa₃-Aa₄-Aa₅, Aa₄-Aa₅ and Aa₅, or is absent. Aa₁, Aa₂, Aa₃, Aa₄, and Aa₅ are selected from the group consisting of amino acids; X is selected from the group consisting of G, A, S and T, or is absent when R is absent; and wherein R' is from 0 to about 59 contiguous amino acids. In a more preferred embodiment, Aa₁ is a glutamine or a conservative substitution thereof, Aa₂ ~~is~~ is a proline or a conservative substitution thereof, Aa₃ ~~is~~ is a lysine or a conservative substitution thereof, Aa₄ ~~is~~ is an aspartic acid or a conservative substitution thereof, or Aa₅ ~~is a~~ is an asparagine or a conservative substitution thereof.

In another embodiment, R' is selected from the group consisting of Aa₁₂-Aa₁₃-Aa₁₄-Aa₁₅-Aa₁₆, Aa₁₂-Aa₁₃-Aa₁₄-Aa₁₅, Aa₁₂-Aa₁₃-Aa₁₄, Aa₁₂-Aa₁₃ and Aa₁₂, wherein Aa₁₂, Aa₁₃, Aa₁₄, Aa₁₅ and Aa₁₆ are selected from the group consisting of amino

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acids. In a preferred embodiment, Aa₁₂ is a cysteine or a conservative substitution thereof, Aa₁₃ is an isoleucine or a conservative substitution thereof, Aa₁₄ is ~~an~~ a glutamine or a conservative substitution thereof, Aa₁₅ is a methionine or a conservative substitution thereof, or Aa₁₆ is a valine or a conservative substitution thereof.

Please replace the paragraph beginning at page 6, line 18 with the following rewritten paragraph:

Figure 6: Effect of pentapeptide DVCQD (SEQ ID NO:28) on the growth of ~~established~~ established KS Y-1 tumors in mice. Mice were implanted with the tumor on day one. Treatment with the peptide was started on the following day at a dose of 50 mg/kg subcutaneously daily. When compared to the control, the tumor volumes were significantly smaller. ~~Ther~~ The arrow marks initiation of daily subcutaneous dosing.

Please replace the paragraph beginning at page 13, line 12 with the following rewritten paragraph:

Another example of algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul, et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (~~http://www.ncbi.nlm.nih.gov/~~). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul, et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs

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containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M⁵, N⁻⁴, and a comparison of both strands.

Please replace the paragraph beginning at page 15, line 24 with the following rewritten paragraph:

The phrase "antibody raised against Saposin B" refers to antibodies that can neutralize the anti-angiogenic activity of ~~Saposin~~ Saposin B or of the active peptides provided herein. The antibodies can be either polyclonal or monoclonal. These antibodies are produced or raised by immunogenically exposing Saposin B to the immune system of an animal able to produce antibodies specific to Saposin B.

Please replace the paragraph beginning at page 18, line 2 with the following rewritten paragraph:

In the Sequence Listing, SEQ ID NO:1 2 corresponds to the amino acid sequence of Saposin B. At position ~~7~~ 2 of this amino acid sequence is an aspartic acid residue. SEQ ID NO:2 1 is the amino acid sequence of full length Prosaposin. SEQ ID NOs:3 and 4 are nucleic acid primers used to amplify the nucleic acid sequence which encodes Saposin B. SEQ ID NOs:4 5 and ~~5~~ 6 are nucleic acid

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primers used to amplify the nucleic acid sequence which encodes Prosaposin. SEQ ID NOs:6 7 and 7 8 are nucleic acid primers used to amplify the nucleic acid sequence which encodes Saposin A. SEQ ID NOs:8 9 and 9 10 are nucleic acid primers used to amplify the nucleic acid sequence which encodes Saposin C. SEQ ID NOs:10 11 and 11 12 are nucleic acid primers used to amplify the nucleic acid sequence which encodes Saposin D.

Please replace the paragraph beginning at page 21, line 9 with the following rewritten paragraph:

The polypeptides of this invention can be obtained from natural sources. Natural sources in this context comprises mammals including, but not limited to, humans. In a preferred embodiment, the polypeptides of this invention are isolated from the body fluids of humans. In a particularly preferred embodiment, the body fluid is urine. In this embodiment, the preferred polypeptide is Saposin B (SEQ ID NO:1 2).

Please replace the paragraph beginning at page 24, line 21 with the following rewritten paragraph:

After the libraries have been created, the colonies must be probed to identify those colonies that contain the DNA of interest. Nucleic acid probes are nucleotide sequences that specifically hybridize under stringent conditions to the desired nucleic acid. Because the amino acid as well as the nucleotide sequence of Saposin B is known, generating probes to isolate clones with desired DNA would be considered routine and is not a critical aspect of this invention. In a preferred embodiment, the probes are chemically synthesized with a DNA synthesizer, amplified using the primers as shown in SEQ ID NOs:3 and 4, and expanded by cloning into a bacterial vector. The probes are then labeled by techniques well

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known in the art and the library is screened. Screening techniques with labeled nucleic acid probes is also well known in the art.

Please replace the paragraph beginning at page 27, line 8 with the following rewritten paragraph:

In a preferred embodiment, the nucleic acid sequences which encode the polypeptides of this invention are amplified with primers that correspond to SEQ ID NOs:3 through 5 6. These primers are specific for Saposin B (SEQ ID NOs:3 and 4) and proSaposin (SEQ ID NOs:5 and 6).

Please replace the paragraph beginning at page 48, line 28 with the following rewritten paragraph:

Example 2: Saposin B ~~Inhibits~~ Inhibits Endothelial Cell Migration

Please replace the paragraph beginning at page 50, line 24 with the following rewritten paragraph:

To determine whether Saposin B was cytotoxic to CD34⁺/Flk-1⁺ progenitors, CD34⁺/Flk-1⁺ cells from cord blood were isolated and plated on fibronectin coated dishes in the presence or absence of 50 ng/mL Saposin B. In the absence of Saposin B, adherent cells were observed to proliferate and mature into spindle shaped endothelial cells. However in the presence of Saposin B, CD34⁺/Flk-1⁺ progenitor ~~growth~~ growth was inhibited.

Please replace the two paragraphs beginning at page 52, line 25 with the following rewritten paragraphs:

To determine if Saposin B polypeptides had anti-angiogenic activity, a series of overlapping polypeptides were synthesized and tested in the KS Y-1 cell

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proliferation assay. The overlapping polypeptides are SEQ ID NOs:13 through 42 43. The results are tabulated in Tables 4 through 8. Cell Proliferation assays in either KS cells or fibroblast. Cells were plated in 48 well plates at equal numbers in appropriate culture medium. Cells were treated with the test compounds at various concentrations on day one, and on day 3. MTT was done on day 5. KS cells were used to represent the activated endothelial cells, while fibroblast cells represent the control cells. The results are remarkable for the lack of toxicity to the fibroblast relative to the activated endothelial cells/KS cells. Similar results were seen in proliferating endothelial cells. These results support the findings of antiangiogenic properties of these test compounds.

In further experiments, KS tumors were allowed to grow for five days before treatment with Saposin B (1 and 5 mg/kg daily at a distal site from the ~~tumor~~ tumor). In control mice, tumors grew to a weight of 555±mg. Inhibition of tumor growth was observed in Saposin B treated mice with weights of excised tumors being approximately 23% that of controls. See Figure 5. Tumors excised at the conclusion of the experiments were also examined for apoptosis, blood vessel vessel density, ~~and mitotic~~ and mitotic index. Saposin B treated tumors showed an increase in apoptosis and decrease in blood vessel density. Thus, contrary to the results in vitro, Saposin B has an inhibitory effect against non-KS tumors.

Please replace the paragraph beginning at page 54, line 9 with the following rewritten paragraph:

Positive results from the polypeptide represented as SEQ ID NO:19 suggested that other, smaller polypeptides within that region would also have activity. The results of cell proliferation assays using smaller polypeptides of SEQ ID NOs:20-31 are tabulated in Table 7.